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DEVELOPMENT AND EVALUATION OF IMMUNOMODULATORS OF HEMOPOIETIC AND IMMUNOLOGIC MECHANISMS

Annual Report

George C. Tsokos, M.D., Ph.D.

October 1, 1988

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

Army Project Order No. 87PP7858

Uniformed Services University
of Health Sciences
4301 Jones Bridge Road
Bethesda, Maryland 20814-4799

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SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION / AVAILABILITY OF REPORT Approved for public release; distribution unlimited
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION Uniformed Services University of Health Sciences 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code) 4301 Jones Bridge Road Bethesda, MD 20814-4799	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING / SPONSORING ORGANIZATION U.S. Army Medical Research & Development Command 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER Army Project Order No. 87PP7858
8c. ADDRESS (City, State, and ZIP Code) Fort Detrick, Frederick, Maryland 21701-5012	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. 62770A; PROJECT NO. 3M6 2770A871; TASK NO. AH; WORK UNIT ACCESSION NO. 363

11. TITLE (Include Security Classification)
Development and Evaluation of Immunomodulators of Hemopoietic and Immunologic Mechanisms

12. PERSONAL AUTHOR(S)
Tsokos, George C.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Annual Report	FROM 6/15/87 TO 9/30/88	1988 October 1	17

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	
06	15		RA I, Mice, IgD, PO, Immunomodulators, Hemopoiesis, Monoclonal Antibodies
06	03		

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

During the first year, 14 agents were tested. The most effective agents, taking both prophylactic and therapeutic afficacy, were xerosin, AVS 2776, and AVS2777.

Interleukin 4 was found to induce, Epstein -Barr Virus infected human B cells to produce IgE. IFNg was found in contrast to suppress IgE production. Thus we have identified two lymphokines which regulate IgE production by virus infected human B cells.

20. DISTRIBUTION / AVAILABILITY OF ABSTRACT ☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT. ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION Unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL Mrs. Virginia M. Miller	22b. TELEPHONE (Include Area Code) 301/663-7325 22c. OFFICE SYMBOL SGRD-RMI-S

DD Form 1473, JUN 86 Previous editions are obsolete. SECURITY CLASSIFICATION OF THIS PAGE

FOREWORD

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

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Hemopoietic and Survival Studies in Immunosuppressed Mice:
As described in the original proposal, studies to evaluate selected USAMRIID AVS agents for hemopoietic effects and for the ability to nonspecifically enhance resistance to opportunistic infections in immunosuppressed hosts were evaluated by Dr. M. L. Patchen at the Armed Forces Radiobiology Research Institute (AFRRI). The ability of various agents to stimulate hemopoiesis at the level of the pluripotent hemopoietic stem cell was evaluated using the endogenous spleen colony-forming unit assay (E-CFU assay). In this assay, the hemopoietic stem cell content of an animal is reduced by exposure to a sublethal dose of irradiation. Experimental drugs are administered either before (prophylactic drug treatment) or after (therapeutic drug treatment) the hemopoietic injury and evaluated for the ability to accelerate hemopoietic regeneration. Increases in E-CFU numbers indicate a drugs's ability to function as a hemopoietic stimulant. The second parameter assayed in studies performed at AFRRI was the ability of the same selected AVS agents to prophylactically or therapeutically enhance survival in severely irradiated mice. Since following radiation doses such as those used in these experiments, death results from opportunistic pathogens, these studies provide an indication of the ability of an agent to enhance nonspecific resistance in an immunocompromised host.

During the first year of this contract 14 USAMRIID AVS agents were evaluated for these parameters. Results of these studies are summarized in the following two tables and four figures. The most effective agents, taking both prophylactic and therapeutic efficacy into account were Xerosin, AVS 2776, and AVS 2777.

BRM DOSAGES

AVS #	ug/kg			mg/kg											
	1.25	2.5	5	2.5	5	10	12.5	20	25	40	50	100	200	300	400
?							▓		▓		▓				
00001											▓	▓	▓		
1758												▓	▓		▓
1968						▓		▓		▓					
2149				▓	▓	▓									
2776													▓	▓	▓
2777													▓	▓	▓
2778													▓	▓	▓
3926							▓		▓		▓				
3927							▓		▓		▓				
3933															
3934											▓	▓	▓	▓	
3960	▓	▓	▓						▓		▓	▓			
4054															

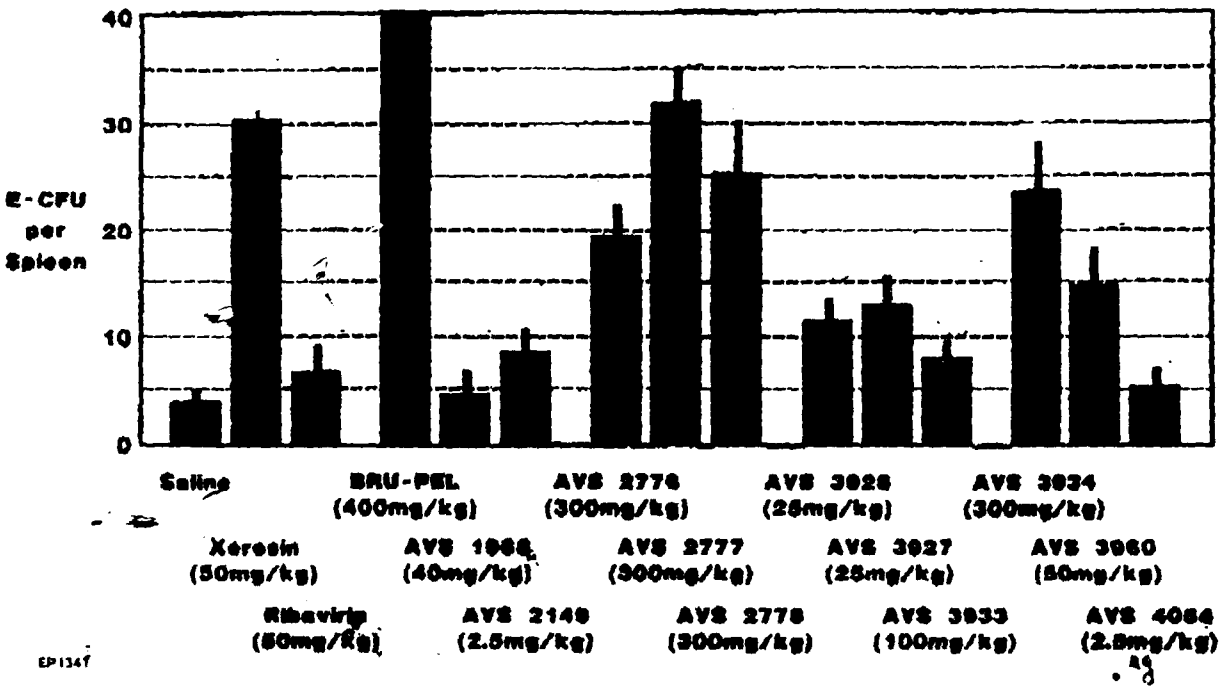
EP1368T

Comparative E-CFU and Survival Enhancing Effects of Tested BRM's (optimal drug doses)

		Prophylactic Treatment		Therapeutic Treatment	
BRM	mg/kg	E-CFU	% Survival	E-CFU	% Survival
Saline	-	4.0 ± 0.9	5	4.0 ± 0.9	40
AVS ?	50.0	30.3 ± 2.2	70	▶40	85
AVS 00001	50.0	6.3 ± 2.8	30	8.0 ± 1.5	35
AVS 1758	400.0	▶40	40	▶40	15
AVS 1968	40.0	4.7 ± 1.6	15	5.7 ± 1.5	45
AVS 2149	2.5	8.8 ± 1.7	25	5.7 ± 1.2	30
AVS 2776	300.0	19.6 ± 2.5	80	24.7 ± 2.8	85
AVS 2777	300.0	32.0 ± 3.2	80	30.5 ± 1.3	90
AVS 2778	300.0	25.3 ± 4.9	90	10.5 ± 1.3	0
AVS 3926	25.0	11.1 ± 2.3	20	17.4 ± 2.3	70
AVS 3927	25.0	8.5 ± 1.4	20	11.6 ± 1.7	70
AVS 3933	100.0	8.0 ± 1.5	50	15.2 ± 2.3	45
AVS 3934	300.0	23.8 ± 4.3	30	10.9 ± 1.3	50
AVS 3960	50.0	14.8 ± 3.3	0	15.5 ± 4.2	20
AVS 4054	2.5(ug/kg)	5.2 ± 1.8	0	15.2 ± 1.3	60

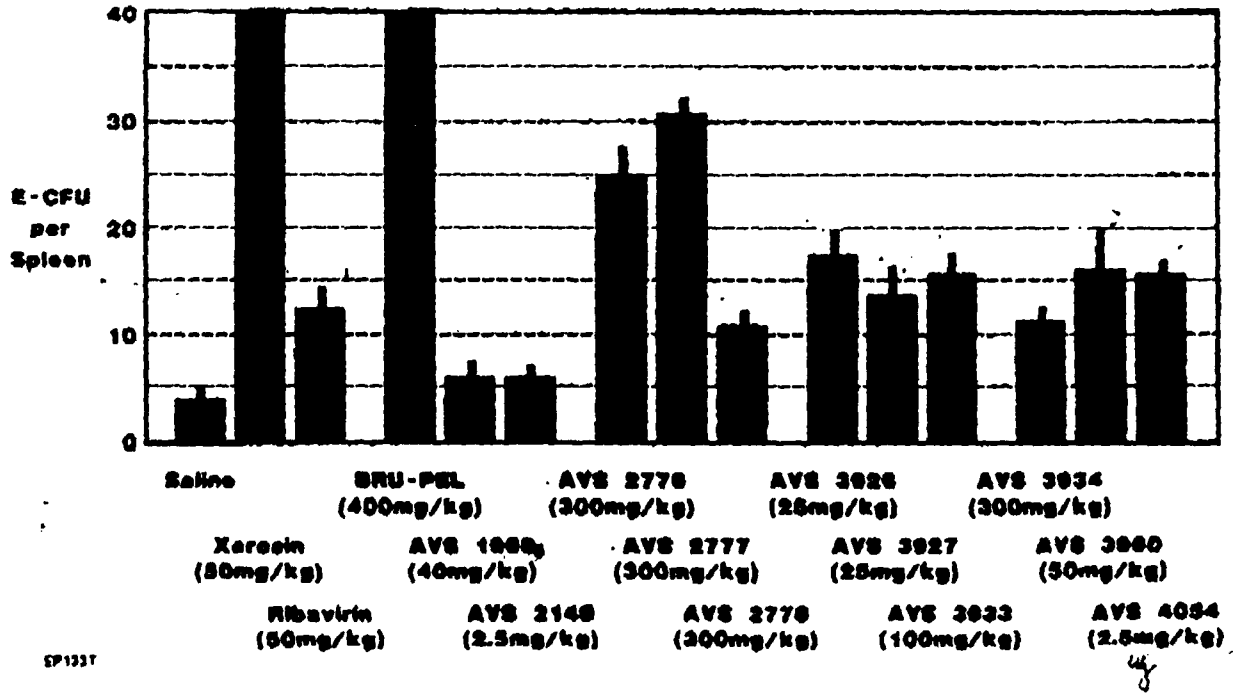
EP1357T

Comparative Effects of Tested BRM's on Hemopoietic Stem Cell Regeneration in C3H/HeN Mice Following 6.5 Gy (Prophylactic Administration)



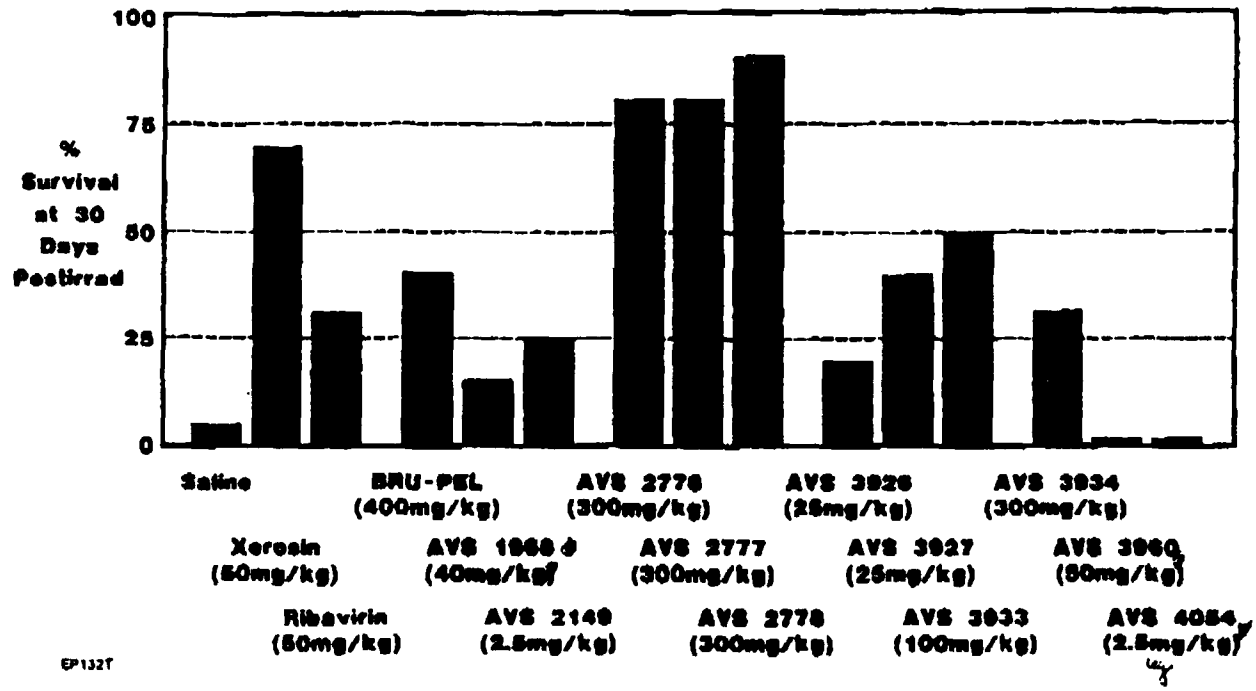
EP1347

Comparative Effects of Tested BRM's on Hemopoietic Stem Cell Regeneration in C3H/HeN Mice Following 6.5 Gy (Therapeutic Administration)



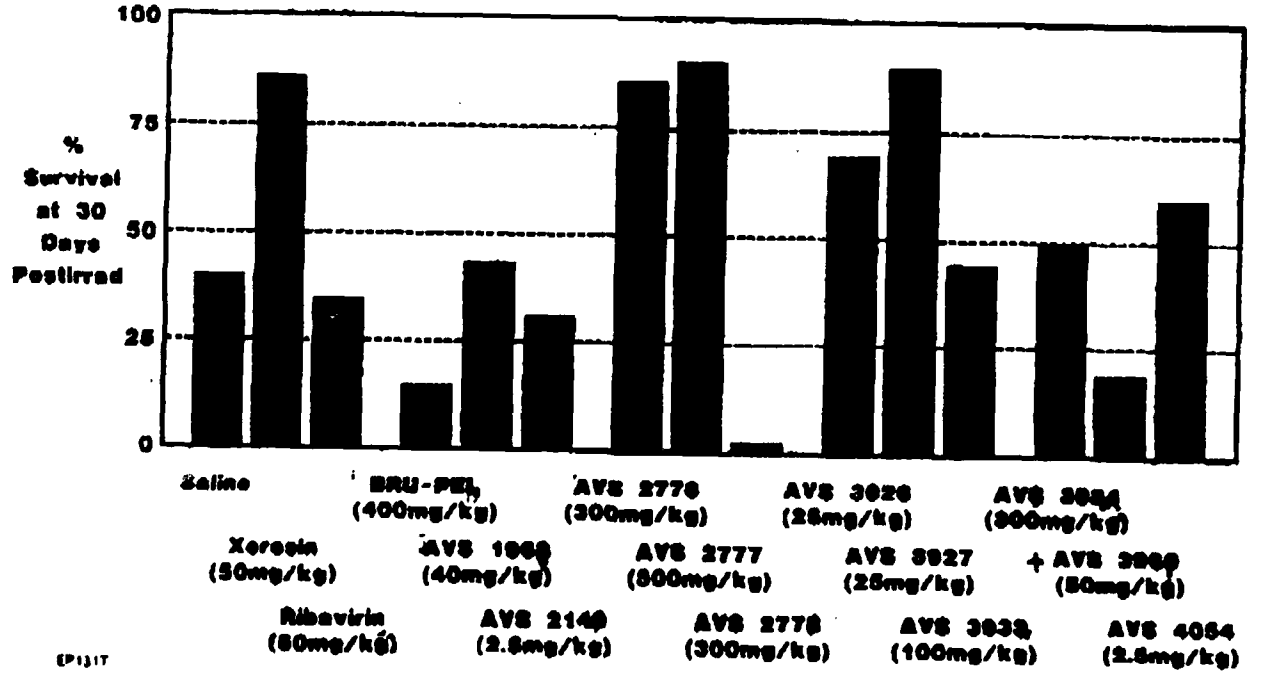
EP1337

Comparative Effects of Tested BRM's on Survival in C3H/HeN Mice Following 8.5 Gy (Prophylactic Administration)



EP132T

Comparative Effects of Tested BRM's on Survival in C3H/HeN Mice Following 8.0 Gy (Therapeutic Administration)



EP131T

Report on Contract #G283BC
Title Development and evaluation of Immunomodulators of hemopoietic and immunologic mechanisms.

B: Development of gene ral and specific immunopotentiators

In our initial proposal we had stated that we were interested in developing general and specific immunopotentiators. Earlier data had shown that injection of mice with goat anti-IgD antibodies leads to a. the appearance of T-lymphocytes bearing receptors for the Fc portion of IgD and b. increase of serum IgD levels; furthermore, injection of IgD antibodies to mice have a general immunopotentiating effect. We hypothesized that we could produce monoclonal IgD antibodies with defined specificities which could be injected into mice and enhance specifically the response to a specific antigen. We injected mice with goat-anti-IgD antibodies and we fused their spleen cells with a myeloma partner and screened for IgD production. During the initial 6 months of the proposal we were able to produce 6 monoclonal IgD antibodies. None of these has specificity for any of the tested antigens. Slow production of IgD monoclonals was due to delays in training a technician and other technical difficulties. At that time point we started considering alternatives towards the production of immunopotentiating agents.

My laboratory is involved in the study of the regulation of B cell function both in normal individuals and in immunocompromised patients. Along these lines we observed that interleukin-4 (IL-4) was capable of inducing the production of IgE by polyclonally stimulated human B cells. We set up a number of experiments to dissect the mechanisms whereby IL-4 leads polyclonally stimulated B cell to the production of IgE. The first task was to find out whether IL-4 can induce human B cells to produce IgE in the absence of other cells (macrophages and T lymphocytes). This was particularly important since IL-4 is known to stimulate T cells. To obtain highly purified human B cells we first subjected the mononuclear cell (MNC) to gradient centrifugation over Sepracell to remove monocytes and treated them with a coctail of monoclonal antibodies, directed against NK, T lymphocytes and macrophages and then rosetted with goat-anti-mouse coated magnetic beads, after which the rosettes were magnetically removed. This procedure yields highly purified (> 5%) B cells. Purified B cells were cultured with Epstein-Barr virus (EBV), a demonstrated T-cell independent, Polyclonal B cell activator, in the presence or absence of IL-4. Table I presents data from a dose response experiment, which shows that IL-4 enhances the production of IgA, IgG and IgM by purified human B cells cultured in the presence of EBV. In the absence of IL-4, IgE cannot be detected in the supernatants of B cells cultured with EBV. If IL-4 is present in the cultures then significant amounts of IgE are secreted (Table II). To ensure that IgE is produced we stained IL-4 and EBV-treated cells for cytoplasmic IgE. Indeed 10 our to 3750 cells stained positive for IgE. No IgE producing cells could be detected among the cells treated with EBV in the absence of IL-4 (Table III). IL-4

had an effect primarily on cell differentiation and immunoglobulin production while it had minimal effect on cell growth (Table IV). We next determined that IL-4 is needed primarily at the initiation of the cultures. Cells cultured with EBV without IL-4 for four days and then with IL4 up to 24 days failed to produce IgE (Fig. 1). Cells had to be cultured with EBV and IL-4 for at least 18 days before IL-4 could be detected (Fig. 2). In the course of these experiments we attempted to define the optimum dosage of IL-4 needed for the production of IgE. One complete experiment, presented in Fig. 3, indicates that the dose response curve is bimodal with one peak at 33 Units or IL-4 and another at 1000 units of IL-4 per ml. We have concluded at this point that IL-4 is able to induce polyclonally activated human B cells to produce IgE.

These experiments are of utmost biological significance; IgE is the main immunoglobulin involved in the defense of organisms against parasitic infections and also in the development of allergic reactions. Little is known about the regulation of the production of IgE by humans. Our in vitro system demonstrates that human B cells can be induced to produce IgE. Our short term goals are to better determine the biology of IgE induction by IL-4. We wish to show that IL-4 has its effect on B cells directly and not through substances secreted by B cells which have been activated by EBV and/or IL-4. Next we want to see whether IL-4 causes IgM^+IgD^+ B cells to switch to IgE producing cells or the expansion of IgE^+ cells. Furthermore, we wish to examine whether B cell surface molecules, besides surface Ig, may participate in the induction of IgE production by IL-4. In the long run we wish to examine the effect of IL-4 and other lymphokines in the regulation of the response of human cells to parasites.

EFFECT OF IL-4 ON IgA,IgG,IgM SECRETION

IL-4 Units/ml	IgA ng/ml	IgG ng/ml	IgM ng/ml
0	191	29	327
10	943	222	4290
33	1061	380	5782
100	1517	397	9406
333	1639	925	10520
1000	898	125	1370

EFFECT OF IL-4 ON IMMUNOGLOBULIN PRODUCTION

IL-4 Units/ml	IgG ng/ml	IgM ng/ml	IgE pg/ml
0	3393	5850	<150
10	4493	5610	<150
100	7842	10614	6167
1000	8427	13489	10018

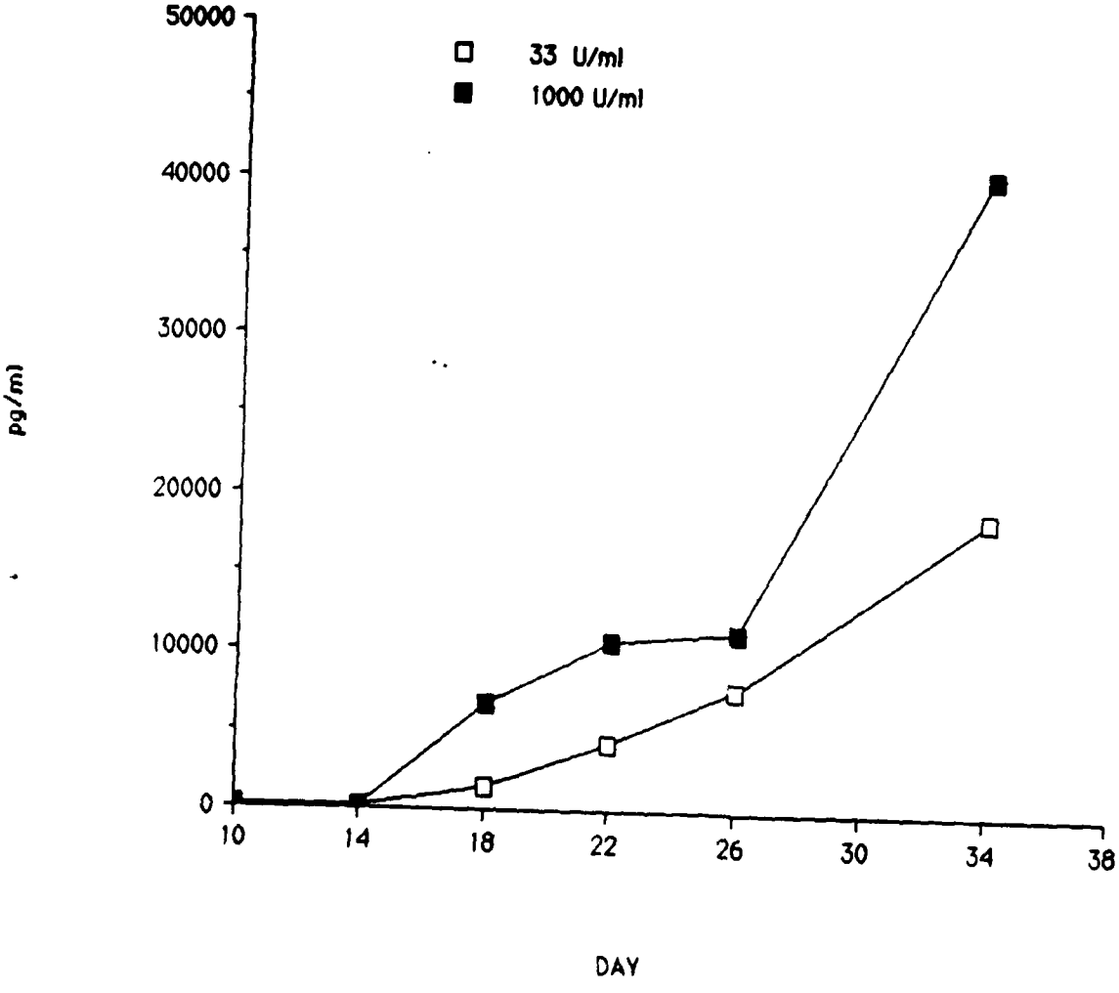
CYTOPLASMIC STAINING

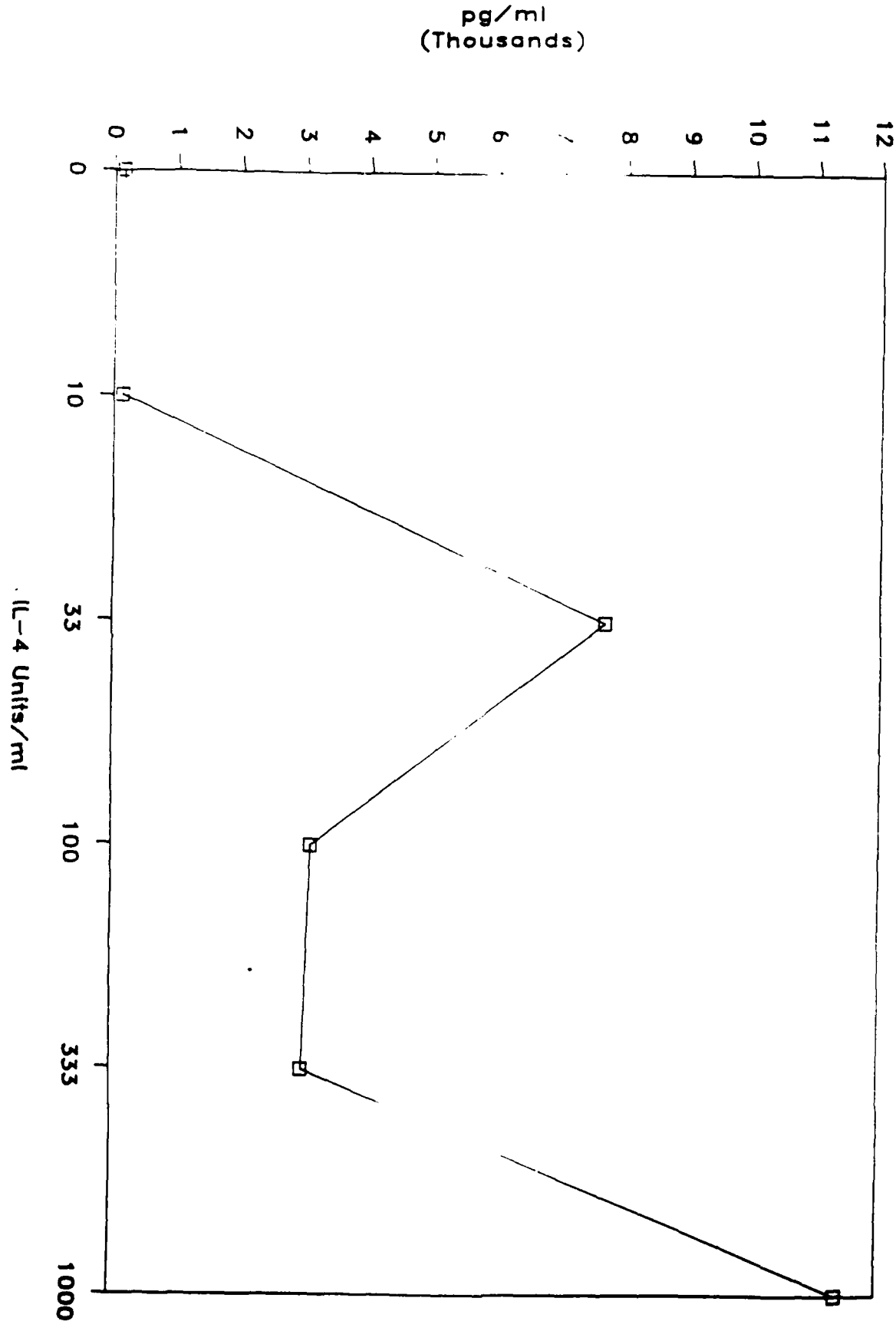
IL-4 Units/ml	IgE cells counted	IgE cells stained	IgM % +
0	6250	0	17
1000	3750	10	41

EFFECT OF IL-4 ON CELL GROWTH

IL-4 Units/ml	Cells/ml X1000	Index of stimulation
0	77	
10	260	3.3
33	360	4.6
100	380	4.9
333	404	5.2
1000	140	1.8

KINETICS OF THE IGE PRODUCTION





DOSE RESPONSE

IGE PRODUCTION AFTER ADDITION OF
IL-4 AT DIFFERENT TIME POINTS